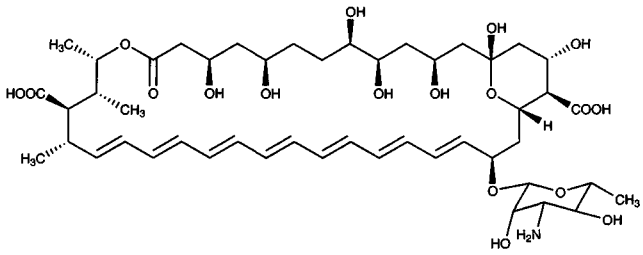
Amphotericin



Molecular formula: $C_{47}H_{73}NO_{17}$
Molecular weight: 924.09
CAS Registry No.: 1397-89-3
Merck Index: 627

SAMPLE

Matrix: CSF
Sample preparation: Condition a BakerBond C18 SPE cartridge with 3 mL MeOH and 3 mL 100 mM pH 9 carbonate buffer. 1 mL CSF + 50 μL 10 μg/mL nystatin in MeOH, vortex briefly, add to the SPE cartridge, wash with 2 mL 100 mM pH 9 carbonate buffer, air dry for 2 min, elute with two 500 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 200 μL MeOH, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:10 mM pH 5 EDTA 35:65
Flow rate: 0.5
Injection volume: 100
Detector: UV 410

CHROMATOGRAM

Retention time: 7.6
Internal standard: nystatin (8.5)
Limit of detection: 0.5 ng/mL

KEY WORDS

dog; human; SPE; pharmacokinetics

REFERENCE

Liu,H.; Davoudi,H.; Last,T. Determination of Amphotericin B in cerebrospinal fluid by solid-phase extraction and liquid chromatography, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1395–1400.

SAMPLE

Matrix: blood
Sample preparation: Add 800 μL cold MeOH to 200 μL serum, mix and centrifuge at 5° at 7000 rpm for 5 min. Filter (Millipore 0.45 μm) the supernatant, inject a 5 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 33 × 4.6 1.5 μm MICRA NPS RP-C18 (Micra Sccientific, Northbrook, IL, USA)
Mobile phase: MeCN:MeOH:50 mM sodium acetate 30:30:40
Flow rate: 0.7
Injection volume: 5
Detector: UV 382

CHROMATOGRAM

Retention time: 2.7
Limit of detection: 0.2 ng
Limit of quantitation: 0.625 ng

KEY WORDS
serum; dog; pharmacokinetics

REFERENCE
Betto,P.; Rajevic,M.; Bossù,E.; Gradoni,L. Improved assay for serum amphotericin-B by fast high performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 1857–1866.

SAMPLE
Matrix: blood
Sample preparation: Extract a 200-300 μL aliquot of whole blood with DMSO:MeOH 2:1 or chloroform:MeCN:DMSO 30:30:40 containing IS. Mix for 30 s, allow the mixture to sit for 1 hour and repeat mixing. Centrifuge, filter (0.45 μm) and inject a 50-100 μL aliquot of the supernatant. (Caution! Chloroform is a carcinogen!)

HPLC VARIABLES
Column: 150 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water adjusted to pH 4.2 with EDTA 36:64
Flow rate: 1
Injection volume: 50-100
Detector: UV 405

CHROMATOGRAM
Internal standard: N-acetyl-amphotericin B
Limit of quantitation: 75 ng/mL

KEY WORDS
pharmacokinetics; whole blood

REFERENCE
Adedoyin,A.; Bernardo,J.F.; Swenson,C.E.; Bolsack,L.E.; Horwith,G.; DeWit,S.; Kelly,E.; Klasterksy,J.; Sculier,J.P.; DeValeriola,D.; Anaissie,E.; Lopez-Berestein,G.; Llanos-Cuentas,A.; Boyle,A.; Branch,R.A. Pharmacokinetic profile of ABELCET (amphotericin B lipid complex injection): combined experience from phase I and phase II studies, *Antimicrob.Agents Chemother.*, **1997**, *41*, 2201–2208.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 600 μL MeOH, vortex, centrifuge at 10500 g for 5 min, inject an 80 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 30 × 4.6 3 μm C18 (Perkin-Elmer)
Mobile phase: MeCN:2.5 mM disodium EDTA 30:70
Flow rate: 1
Injection volume: 80
Detector: UV 405

CHROMATOGRAM
Retention time: 1.5
Limit of detection: 50 ng/mL

KEY WORDS
serum

REFERENCE
Lopez-Galera,R.; Pou-Clave,L.; Pascual-Mostaza,C. Determination of amphotericin B in human serum by liquid chromatography, *J.Chromatogr.B*, **1995**, *674*, 298–300.

SAMPLE
Matrix: blood
Sample preparation: Deproteinize serum with MeOH, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 30 mm long C18
Mobile phase: MeCN:2.5 mM disodium EDTA 30:70
Flow rate: 1
Detector: UV 405

CHROMATOGRAM
Retention time: 1.5
Limit of quantitation: 50 ng/mL

KEY WORDS
pharmacokinetics; serum

REFERENCE
Ayestarán,A.; López,R.M.; Montoro,J.B.; Estíbalez,A.; Pou,L.; Julià,A.; López,A.; Pascual,B. Pharmacokinetics of conventional formulation versus fat emulsion formulation of amphotericin B in a group of patients with neutropenia, *Antimicrob.Agents Chemother.*, **1996**, *40*, 609–612.

SAMPLE
Matrix: blood, fibrin clot
Sample preparation: Digest fibrin clots with trypsin (1:1). 250 μL Serum or digested fibrin clot + 250 μL MeCN, vortex for 1 min, let stand at room temperature for 10 min, centrifuge at 2000 g, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 4.6 10 μm Spherisorb C18-ODS2
Mobile phase: MeOH:5 mM EDTA 70:30 adjusted to pH 7.8 with 1 M ammonium hydroxide
Column temperature: 40
Flow rate: 1
Injection volume: 100
Detector: UV 385

CHROMATOGRAM
Retention time: 4.7
Limit of quantitation: 10 ng/mL

KEY WORDS
rabbit; serum; human; pharmacokinetics

REFERENCE
Bouley,M.; Tod,M.; Chavanet,P.; Petitjean,O. The penetration of amphotericin B from an Intralipid formulation into fibrin loci in a rabbit model of candidiasis, *Biopharm.Drug Dispos.*, **1994**, *15*, 485–492.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. Mix 100 μL plasma with 300 μL MeOH, heat in a 50° water bath for 15 min, cool at room temperature for 5 min, centrifuge at 9500 g for 10 min. Inject a 50 μL aliquot. Tissue. Mix 500 mg rat tissue with 4.5 mL MeOH and 500 μL 10 mM pH 7.4 phosphate buffer. Homogenize for 5 min, vortex for 5 min, centrifuge until the supernatant is clear, inject a 120 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS (plasma) or 216 × 4.6 10 μm C18 (Whatman) (tissue)
Mobile phase: MeOH:1 mM disodium EDTA containing 82 mM triethylamine and 96 mM phosphoric acid:deionized water 8:1:1.25 (plasma) or MeCN:10 mM sodium acetate 39.4:60.6
Column temperature: 35.5 (tissue)
Flow rate: 0.8 (plasma), 1 (tissue)
Injection volume: 50 (plasma), 120 (tissue)
Detector: UV 382

CHROMATOGRAM
Limit of quantitation: 50 ng/mL (plasma), 500 ng/g (tissue)

KEY WORDS
plasma; rat; pharmacokinetics; brain; kidney; liver; lung; spleen

REFERENCE
Boswell,G.W.; Bekersky,I.; Buell,D.; Hiles,R.; Walsh,T.J. Toxicological profile and pharmacokinetics of a unilamellar liposomal vesicle formulation of amphotericin B in rats, *Antimicrob.Agents Chemother.*, **1998**, *42*, 263–268.

SAMPLE
Matrix: blood, tissue
Sample preparation: Serum. 50-150 μL Serum + 50 μL 25 μg/mL IS in MeOH + 2 mL MeOH:acetic acid 90:10, vortex for 30 s, leave in the dark for 1 h, centrifuge at 1000 g for 10 min, decant, filter (0.45 μm), inject a 100 μL aliquot. Tissue. 100-200 mg Tissue + 50 μL 25 μg/mL IS + 500 μL 1 mM pH 7.4 phosphate buffer, vortex, homogenize using a manual glass homogenizer, add 2 mL MeOH:acetic acid 90:10, vortex for 30 s, leave in the dark for 1 h, centrifuge at 1000 g for 10 min, decant, filter (0.45 μm), inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 2 Alltech C18
Column: 300 × 3.9 10 μm μBondapak RP-C18
Mobile phase: MeCN:10 mM pH 4.0 acetate buffer 37:63
Flow rate: 1 for 6 min, then 2
Injection volume: 100
Detector: UV 383

CHROMATOGRAM
Retention time: 15
Internal standard: natamycin (6) (UV 303)
Limit of detection: 100 ng/mL
Limit of quantitation: 1 μg/mL

KEY WORDS
serum; lung; liver; mouse; pharmacokinetics

REFERENCE
Polikandritou Lambros,M.; Abbas,S.A.; Bourne,D.W.A. New high-performance liquid chromatographic method for amphotericin B analysis using an internal method, *J.Chromatogr.B*, **1996**, *685*, 135–140.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize 0.5 g tissue with 1 mL MeOH for 1 min. 100 μL Serum or tissue homogenate + 100 μL cold MeCN, vortex for 10 s, centrifuge at 11800 g for 2 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelco LC-1
Mobile phase: MeOH:5 mM EDTA buffer 65:35

CHROMATOGRAM
Limit of detection: 50 ng/mL

KEY WORDS
serum; rat; kidney; liver; lung

REFERENCE
Wasan,K.M.; Vadiei,K.; Lopez-Berestein,G.; Luke,D.R. Pharmacokinetics, tissue distribution, and toxicity of free and liposomal amphotericin B in diabetic rats, *J.Infect.Dis.*, **1990**, *161*, 562–566.

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize 0.5 g tissue in 4.5 mL MeOH. Extract tissue homogenate or 0.5 mL plasma using a 1 mL Bond-Elut C18 SPE cartridge.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:2.5 mM disodium EDTA 45:55
Detector: UV 382

CHROMATOGRAM
Limit of detection: ≤25 ng/g, ≤5 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics; liver; lung; kidney; spleen; heart; skin; lymph nodes; adrenal glands; thyroid; pancreas; testes; ileum; SPE

REFERENCE
Fielding,R.M.; Smith,P.C.; Wang,L.H.; Porter,J.; Guo,L.S. Comparative pharmacokinetics of amphotericin B after administration of a novel colloidal delivery system, ABCD, and a conventional formulation to rats, *Antimicrob.Agents Chemother.*, **1991**, *35*, 1208–1213.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Condition a 1 mL 100 mg Bond-Elut C18 SPE cartridge with 1-2 mL MeOH, with 1-2 mL water, and with 3 mL 10 mM pH 7.4 phosphate buffer. 0.5 mL Blood or 0.3-0.5 g tissue + 0.5 mL 10 mM pH 7.4 phosphate buffer, homogenize (Polytron homogenizer) for 5-10 s, add 4 mL MeOH, vortex for 30 s, centrifuge at 2000 g for 10 min. Add 0.5 mL plasma, 1-2 mL urine, or 0.5-2 mL supernatant from blood or tissue to 4 mL 10 mM pH 7.4 phosphate buffer, add this mixture to the SPE cartridge at < 1 mL/min, wash with 3 mL MeOH:10 mM pH 7.4 phosphate buffer 40:60, centrifuge SPE cartridge at 2000 g for 2-3 min, elute with 0.75 (plasma) or 1 (others) mL MeCN:2.5 mM disodium EDTA 60:40 (plasma) or 50:50 (others), centrifuge to remove the last of the eluate, inject a 100 μL aliquot of the eluate.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:2.5 mM disodium EDTA 45:55
Flow rate: 1
Injection volume: 100
Detector: UV 382

CHROMATOGRAM

Retention time: 7

Limit of detection: ≤25 ng/mL (blood), 2.5 ng/mL (urine), 50 ng/g (tissue), ≤5 ng/mL (plasma)

KEY WORDS

plasma; SPE; rat; liver; kidney; lung; spleen; heart; brain; muscle; pharmacokinetics; stability

REFERENCE

Wang,L.H.; Smith,P.C.; Anderson,K.L.; Fielding,R.M. High-performance liquid chromatographic analysis of amphotericin B in plasma, blood, urine and tissues for pharmacokinetic and tissue distribution studies, *J.Chromatogr.*, **1992**, *579*, 259–268.

SAMPLE

Matrix: blood, urine

Sample preparation: Serum. 100 μL Serum + 300 μL 160 ng/mL IS in ice-cold MeOH, vortex, centrifuge. Remove the supernatant and concentrate it under a stream of nitrogen at room temperature, reconstitute the residue in 150 μL MeOH, inject an aliquot. Urine. Dilute urine. 100 μL Diluted urine + 300 μL 160 ng/mL IS in ice-cold MeOH, vortex, inject an aliquot.

HPLC VARIABLES

Column: Nova-Pak C18

Mobile phase: MeCN:4 mM pH 7.0 Na_2HPO_4/KH_2PO_4 buffer 31:69

Flow rate: 1.2

Detector: UV 405

CHROMATOGRAM

Retention time: 3.5

Internal standard: 1-amino-4-nitronaphthalene (5.7)

Limit of quantitation: 22 ng/mL

KEY WORDS

rat; serum; protect from light; pharmacokinetics

REFERENCE

Chow,H.-H.; Wu,Y.; Mayersohn,M. Pharmacokinetics of amphotericin B in rats as a function of dose following constant-rate intravenous infusion, *Biopharm.Drug Dispos.*, **1995**, *16*, 461–473.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 *g* for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 *g* for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 346.2

CHROMATOGRAM
Retention time: 15.718

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bronchoalveolar lavage fluid
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with two 3 mL portions of MeCN and two 3 mL portions of 10 mM pH 7.4 sodium acetate buffer. Mix the total volume of bronchoaspiration or bronchoalveolar lavage fluid with an equal volume of MeOH for 1 min, centrifuge at 3000 rpm for 20 min, combine the supernatant with an equal volume (?) of 10 mM pH 7.4 sodium acetate buffer, add to the SPE cartridge, wash five times with 3 mL portions of MeOH:10 mM pH 7.4 sodium acetate buffer 50:50, elute with two 1.5 mL portions of MeOH. Evaporate the eluate to dryness under nitrogen, reconstitute the residue in 400 μL MeOH, vortex for 15 s, inject an aliquot.

HPLC VARIABLES
Column: 30 × 4.6 3 μm Perkin-Elmer ODS
Mobile phase: MeCN:2.5 mM EDTA disodium dihydrate 30:70
Flow rate: 1
Injection volume: 80
Detector: UV 405

CHROMATOGRAM
Retention time: 1.5
Limit of detection: 50 ng/mL

KEY WORDS
SPE

REFERENCE
Lopez,R.; Pou,L.; Andres,I.; Monforte,V.; Roman,A.; Pascual,C. Amphotericin B determination in respiratory secretions by reversed-phase liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 135–139.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1 mL samples to 5 or 10 mL with 5% dextrose, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Beckman ODS C18
Mobile phase: MeCN:MeOH:buffer 37:18:45 (Buffer was 50 mM sodium acetate and 3 mM disodium EDTA adjusted to pH 5.0 with glacial acetic acid.)
Flow rate: 1.2
Injection volume: 20

Detector: UV 405

CHROMATOGRAM
Retention time: 7.8
Limit of detection: 625 ng/mL

OTHER SUBSTANCES
Simultaneous: amphotericin A, amphotericin X

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Kintzel,P.E.; Kennedy,P.E. Stability of amphotericin B in 5% dextrose injection at concentrations used for administration through a central venous line, *Am.J.Hosp.Pharm.*, **1991**, *48*, 283–285.

SAMPLE
Matrix: formulations
Sample preparation: Directly inject a 20 μL aliquot.

HPLC VARIABLES
Column: 10 μm Radial-Pak μBondapak C18
Mobile phase: MeOH:MeCN:2.5 mM EDTA 50:35:20
Flow rate: 1.8
Injection volume: 20
Detector: UV 405

CHROMATOGRAM
Retention time: 5.8

KEY WORDS
injections; 5% dextrose; stability-indicating

REFERENCE
Mitrano,F.P.; Outman,W.R.; Baptista,R.J.; Palombo,J.D. Chemical and visual stability of amphotericin B in 5% dextrose injection stored at 4 degrees C for 35 days, *Am.J.Hosp.Pharm.*, **1991**, *48*, 2635–2637.

SAMPLE
Matrix: formulations
Sample preparation: 100 μL Liposomal preparation + 100 μL MeOH, vortex for 10 s, centrifuge at 13000 g for 2 min, inject a 75 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelco LC-1
Mobile phase: MeOH:0.005% EDTA 65:35
Flow rate: 2
Injection volume: 75
Detector: UV 405

CHROMATOGRAM
Limit of detection: 50 ng/mL

KEY WORDS
liposomal preparations

REFERENCE

Wasan,K.M.; Morton,R.E.; Rosenblum,M.G.; Lopez-Berestein,G. Decreased toxicity of liposomal amphotericin B due to association of amphotericin B with high-density lipoproteins: Role of lipid transfer protein, *J.Pharm.Sci.*, **1994**, *83*, 1006–1010.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in DMSO to 10 mg/mL, dilute 1:20 with MeOH.

HPLC VARIABLES

Column: 250 × 4.6 10 μm μBondapak C18
Mobile phase: MeCN:50 mM phosphate buffer (pH 3.5-8.1) 30:70 to 35:65
Flow rate: 0.4-2
Detector: UV 313

OTHER SUBSTANCES

Simultaneous: nystatin

KEY WORDS

for amphotericin A

REFERENCE

Aszalos,A.; Bax,A.; Burlinson,N.; Roller,P.; McNeal,C. Physico-chemical and microbiological comparison of nystatin, amphotericin A and amphotericin B, and structure of amphotericin A, *J.Antibiot.(Tokyo)*, **1985**, *38*, 1699–1713.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 15 mM, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 50 × 4.6 3 μm Econosphere C18
Mobile phase: MeOH:buffer 65:35 (Buffer was 6.3 g NaH_2PO_4 in 1 L water, adjust pH to 2.6 with phosphoric acid.)
Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 407

CHROMATOGRAM

Retention time: 10

REFERENCE

Backes,B.J.; Rychnovsky,S.D. A reverse-phase HPLC assay for measuring the interaction of polyene macrolide antifungal agents with sterols, *Anal.Biochem.*, **1992**, *205*, 96–99.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: C18
Mobile phase: MeOH:DMF 20:80
Flow rate: 1.5
Injection volume: 20
Detector: UV 280

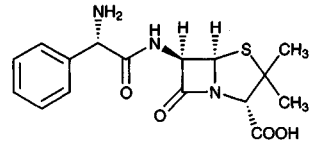
CHROMATOGRAM
Retention time: 2.2

OTHER SUBSTANCES
Simultaneous: cefpirome

KEY WORDS
stability-indicating; protect from light

REFERENCE
Allen,L.V.,Jr.; Stiles,M.L.; Prince,S.J.; Sylvestri,M.F. Stability of cefpirome sulfate in the presence of commonly used intensive care drugs during simulated Y-site injection, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2427–2433.

Ampicillin



Molecular formula: $C_{16}H_{19}N_3O_4S$
Molecular weight: 349.41
CAS Registry No.: 69-53-4 (anhydrous), 32388-53-7 (monohydrate), 23277-71-6 (K salt), 7177-48-2 (trihydrate), 69-52-3 (Na salt)
Merck Index: 628
Lednicer No.: 1 413, 2 437, 4 179

SAMPLE

Matrix: blood, tissue, urine
Sample preparation: Plasma. 50 μL Plasma + 50 μL IS solution + 50 μL MeCN, mix for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot of the supernatant. Urine. 100 μL IS solution + 200 μL MeCN + 100 μL urine, mix for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot. Tissue. Weight out finely chopped tissue and suspend it in 200 μL water. Add 100 μL 100 μg/mL IS, sonicate for 60 s. Add 200 μL MeCN, vortex for 30 s, centrifuge at 10000 g for 15 min. Inject an aliquot.

HPLC VARIABLES

Guard column: 15 × 3.2 7 μm Newguard C18 (Alltech)
Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: MeCN:50 mM pH 5.0 sodium dihydrogen phosphate 10:90
Flow rate: 1.0
Detector: UV 215

CHROMATOGRAM

Retention time: 4.3
Internal standard: cefotaxime (11.6)
Limit of quantitation: 500 ng/mL (plasma), 1 μg/mL (urine), 2.5 μg/g (tissue)

KEY WORDS

plasma; muscle; rat; pharmacokinetics

REFERENCE

Cross,S.E.; Thompson,M.J.; Roberts,M.S. Distribution of systemically administered ampicillin, benzylpenicillin, and flucloxacillin in excisional wounds in diabetic and normal rats and effects of local topical vasodilator treatment, *Antimicrob.Agents Chemother.*, **1996**, *40*, 1703–1710.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.827

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Capsules. Dissolve the powder from five capsules and the capsule shells in water, dilute to 1 L with water, filter an aliquot of the solution (0.2 μm), dilute 10-fold with water, inject an aliquot. Syrup. Reconstitute the syrup powder, dilute 200-fold with water, filter, inject an aliquot. Neonatal suspension. Reconstitute the powder, dilute 800-fold with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 100 × 2.0 3 μm Hypersil ODS
Mobile phase: MeCN:20 mM phosphate buffer 15:85 containing 100 mM sodium dodecyl sulfate, adjusted to pH 2.0 with orthophosphoric acid
Flow rate: 0.4
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 5.2

OTHER SUBSTANCES
Simultaneous: degradation products, cloxacillin

KEY WORDS
capsules; suspensions; syrup

REFERENCE
Shakoor,O.; Taylor,R.B. Analysis of ampicillin, cloxacillin and their related substances in capsules, syrups and suspensions by high-performance liquid chromatography, *Analyst*, **1996**, *121*, 1473–1477.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 18.5 min), evaporate to <1 mL under reduced pressure, add 200 μL 10 mM KH_2PO_4 containing 10 mM phosphoric acid and 10 mM

sodium decanesulfonate, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:buffer 35:65 (Buffer was 10 mM phosphoric acid containing 5 mM potassium dihydrogen phosphate and 5 mM sodium dodecyl sulfate.)
Flow rate: 1
Injection volume: 200
Detector: UV 215

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL 500 mg Baker-10 C18 SPE cartridge (J.T. Baker) with 3 mL MeOH and 3 mL distilled water. Add 20 mL MeCN to 10 mL milk, vortex for 1 min, centrifuge at 1500 g for 10 min, concentrate the supernatant to 2-3 mL on a rotary evaporator at 40°, add to the SPE cartridge, dry the cartridge under reduced pressure for 3 min, elute with 1 mL MeOH, filter (0.45 μm) the eluate, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Kaseisorb LC ODS-300-5 (Tokyo Kasei)
Mobile phase: MeCN:MeOH:50 mM KH_2PO_4 buffer 20:10:80 containing 5 mM sodium 1-decanesulfonate, adjusted to pH 3.5 with concentrated phosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 30 ng/mL

OTHER SUBSTANCES
Extracted: cloxacillin, dicloxacillin, nafcillin, penicillin G

KEY WORDS
SPE

REFERENCE
Takeba,K.; Fujinuma,K.; Miyazaki,T.; Nakazawa,H. Simultaneous determination of β-lactam antibiotics in milk by ion-pair liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 205–211.

SAMPLE
Matrix: milk
Sample preparation: Condition a 3 mL 500 mg Bond Elut C18 SPE cartridge with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl, do not allow to go dry. 5 mL Milk + 500 μL 30% trichloroacetic acid, vortex thoroughly for 10 s, centrifuge at 0° at 3300 g for 30 min. Remove the liquid supernatant and add it to 100 μL 4 M NaOH (to pH 5.2), vortex for 10 s, add 1 mL 20% NaCl, vortex for 10 s, add to the SPE cartridge at 3 mL/min, wash with 1 mL 2% NaCl, wash with 1 mL water, elute with 1 mL MeCN:100 mM pH 6.5 phosphate buffer 40:60. Add 20 μL 2 M NaOH to the eluate and vortex for 10 s (pH 8), add 10 μL 200 mM acetic anhydride in MeCN, let stand for 3 min, add 500 μL reagent,

vortex, heat at 65° for 10 min, cool to room temperature, inject a 200 μL aliquot. (Prepare reagent by dissolving 13.78 g 1,2,4-triazole in 60 mL water, add 10 mL 100 mM mercuric chloride solution, mix, adjust pH to 9.0 ± 0.5 with 4 M NaOH, make up to 100 mL with water.)

HPLC VARIABLES

Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: MeCN:MeOH:buffer 18:12:70 (Prepare buffer by dissolving 4.969 g NaH_2PO_4, 10.139 g $Na_2HPO_4.2H_2O$, 3.894 g sodium thiosulfate pentahydrate, and 6.791 g tetrabutylammonium hydrogen sulfate in 800 mL water, make up to 1 L with water.)
Flow rate: 0.8
Injection volume: 200
Detector: UV 325

CHROMATOGRAM

Retention time: 16
Limit of detection: 3 ng/mL
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Simultaneous: amoxicillin
Noninterfering: cloxacillin, oxacillin, penicillin G

KEY WORDS

derivatization; SPE; cow

REFERENCE

Verdon,E.; Couedor,P. Determination of ampicillin residues in milk by ion-pair reversed phase high performance liquid chromatography after precolumn derivatization, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1201–1207.

SAMPLE

Matrix: milk
Sample preparation: Condition a 500 mg tC18 SPE cartridge (Waters) with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl. Centrifuge 30 mL milk at 1500 g for 10 min. Dilute a 10 mL portion of the defatted milk with 20 mL water, add 200 μL 2 μg/mL penicillin V in pH 9.0 buffer, add 6 mL 170 mM sulfuric acid, add 5.6 mL 5% sodium tungstate, shake vigorously for 1 min, allow to stand for 5 min, check that the pH is in the range 4.6-4.8 (if it is outside this range start again using a different volume of sodium tungstate solution), centrifuge at 1500 g for 10 min, adjust the pH of the supernatant to 8.1-8.2 with 5 M and 0.1 M NaOH, filter (glass fiber) the clear liquid phase. Pass the filtrate through the SPE cartridge at 2 mL/min, wash with 2 mL water, dry by pulling air through the cartridge for 1 min, elute with 2 mL MeCN. Add 150 μL pH 9.0 buffer to the eluate and evaporate to about 100 μL under a stream of nitrogen at 45-50°, add 400 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, use 500 μL water to transfer the mixture to a separatory funnel, add 20 mL dichloromethane, add 5 mL pH 2.45 buffer, shake for 1 min, let stand for no more than 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35-40°, dissolve the residue in 500 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, add 450 μL reagent II, vortex for 1 min, heat at 55 ± 1° for 30 min, cool, filter (0.45 μm), inject a 150 μL aliquot. (Prepare pH 9.0 buffer by dissolving 0.34 g KH_2PO_4 in water, adjusting the pH to 9.0 with NaOH, and making up to 100 mL with water. Prepare pH 2.45 buffer by dissolving 2.72 g KH_2PO_4 in water, adjusting the pH to 2.45 with phosphoric acid, and making up to 100 mL with water. Prepare reagent 1 by dissolving 1.13 g benzoic anhydride in MeCN, make up to 25 mL with MeCN. Prepare reagent II by dissolving 6.905 g 1,2,4-triazole in 30 mL water and adding 5 mL 26 mM mercuric chloride in water, adjust pH to 9.0 ± 0.05 with 5 M NaOH,

make up to 50 mL. Prepare reagents I and II 1-4 h before use. Silanize glassware with dichlorodimethylsilane.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A as MeCN:buffer 10:90. B was MeCN:buffer 30:70. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 13 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 5 min. (Prepare buffer by dissolving 9.938 g Na_2HPO_4, 17.938 g $NaH_2PO_4.H_2O$, and 4.964 g sodium thiosulfate in water, make up to 2 L with water, pH 6.5.)
Column temperature: 30
Flow rate: 1
Injection volume: 150
Detector: UV 323

CHROMATOGRAM
Retention time: 32.5
Internal standard: penicillin V (28.5)
Limit of detection: 1.5 ng/mL
Limit of quantitation: 2.2 ng/mL

OTHER SUBSTANCES
Extracted: amoxicillin, cloxacillin, dicloxacillin, oxacillin, penicillin G

KEY WORDS
derivatization; cow; SPE

REFERENCE
Sorensen,L.K.; Rasmussen,B.M.; Boison,J.O.; Keng,L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high-performance liquid chromatographic method, *J.Chromatogr.B*, **1997**, *694*, 383–391.

SAMPLE
Matrix: perfusate
Sample preparation: Vortex perfusate, centrifuge at 11600 g for 5 min, inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 20 × 2 5 μm Hypersil ODS
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:buffer 30:70 (Buffer was 50 mM KH_2PO_4 containing 0.1% triethylamine adjusted to pH 3 with orthophosphoric acid.)
Flow rate: 1
Injection volume: 100
Detector: UV 229

CHROMATOGRAM
Retention time: 5.6
Limit of detection: 20 ng/mL
Limit of quantitation: 100 ng/mL

REFERENCE
Erah,P.O.; Barrett,D.A.; Shaw,P.N. Reversed-phase high-performance liquid chromatographic assay methods for the analysis of a range of penicillins in in vitro permeation studies, *J.Chromatogr.B*, **1998**, *705*, 63–69.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeCN:1 M acetic acid:1 M KH_2PO_4:water 8:0.1:1:90.9
Flow rate: 1
Detector: UV 201

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 500 mg Isolute SCX SPE cartridge (Jones Chromatography, Hengoed, UK) with MeOH and water. Condition a 100 mg PGC (porous graphitic carbon) SPE cartridge (Shandon, Runcorn, UK) with acetone and pH 7.7 borate buffer. Add 20 mL water to 5 g tissue, homogenize, add 5 mL 170 mM sulfuric acid and 5 mL 5% aqueous sodium tungstate, mix well, centrifuge at 14000 g for 5 min. Discard pellet, add six drops orthophosphoric acid to the supernatant to adjust the pH to 2-2.5. Add it to the SCX SPE cartridge, allow to flow through the cartridge under vacuum at 2 mL/min, wash with 5 mL 10 mM sulfuric acid, elute with 10 mL pH 7.7 borate buffer. Add the eluate to the PGC SPE cartridge. Wash with 5 mL water, place in-line filter (0.2 μm, Anotop) below cartridge, elute with 20 mL acetone. Evaporate to dryness. Add 500 μL water to the dry residue, add 20 μL 2% acetic anhydride in MeCN and let stand for 3 min. Add 500 μL triazole/mercuric chloride derivatizing reagent and heat the mixture at 65° for 20 min. Inject a 100 μL aliquot. (Borate buffer was 200 mM boric acid adjusted to pH 7.7 with 40% NaOH solution. Triazole/mercuric chloride reagent was prepared by mixing 34.45 g 1,2,4-triazole with 150 mL water and 25 mL 10 mM mercuric chloride, adjusted to pH 9.0 with 1 M NaOH and made up to 250 mL with water.) GL –5 μm Kromasil KR 100 C8 (Hichrom)

HPLC VARIABLES
Column: 250 × 3.2 5 μm Kromasil KR 100 C8 (Hichrom)
Mobile phase: MeCN:buffer 20:80 (Buffer was 15 mM potassium dihydrogen phosphate and 15 mM sodium thiosulfate)
Flow rate: 0.55
Injection volume: 100
Detector: UV 325

CHROMATOGRAM
Retention time: 24.5
Limit of detection: 5 μg/kg (muscle)
Limit of quantitation: 50 μg/kg (muscle), 100 μg/kg (liver)

OTHER SUBSTANCES
Extracted: amoxicillin

KEY WORDS
SPE; cow; liver; muscle; derivatization

REFERENCE
Rose,M.D.; Tarbin,J.; Farrington,W.H.; Shearer,G. Determination of penicillins in animal tissues at trace residue concentrations: II. Determination of amoxicillin and ampicillin in liver and muscle using cation exchange and porous graphitic carbon solid phase extraction and high-performance liquid chromatography, *Food Addit.Contam.*, **1997**, *14*, 127–133.

SAMPLE
Matrix: tissue

Sample preparation: Homogenize (Ultra-turrax T25) 5 g muscle with 14 mL 10 mM pH 4.5 sodium phosphate buffer at 10000 rpm for 2 min, add 1 mL 75% trichloroacetic acid in water, shake vigorously for 30 s, centrifuge at 3500 g for 10 min, filter (paper) the supernatant. Remove a 1 mL aliquot of the filtrate, add 200 μL 20% trichloroacetic acid in water, add 200 μL 7% formaldehyde in water, vortex for 20 s, heat at 100° for 30 min, cool to room temperature, make up to 2 mL with MeCN:water 20:80, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Prodigy ODS-3 (Phenomenex)
Mobile phase: MeCN:20 mM pH 3.5 KH_2PO_4 buffer 25:75
Flow rate: 1
Injection volume: 100
Detector: F ex 346 em 422

CHROMATOGRAM

Retention time: 12.5
Limit of detection: 0.6 ng/g
Limit of quantitation: 1.5 ng/g

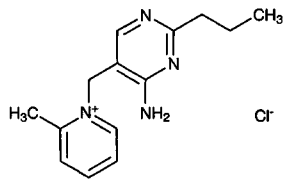
KEY WORDS

derivatization; muscle; cow; pig; chicken; fish; catfish

REFERENCE

Luo,W.; Ang,C.Y.W.; Thompson,H.C.,Jr. Rapid method for the determination of ampicillin residues in animal muscle tissues by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.B*, **1997**, *694*, 401–407.

Amprolium



Molecular formula: $C_{14}H_{19}ClN_4$
Molecular weight: 278.78
CAS Registry No.: 121-25-5
Merck Index: 631
Lednicer No.: 1 264

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 100 μL 100 ng/mL IS in 330 mM perchloric acid + 500 μL 330 mM perchloric acid, vortex for 30 s, centrifuge at 2150 g for 10 min. Remove the supernatant and allow it to stand for 3 h, inject a 30 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: Sumipax PG-ODS-filter (Sumika, Osaka)
Column: 250 × 4.6 5 μm Capcell pack C18 UG-120 (Shiseido, Tokyo)
Mobile phase: MeCN:200 mM KH_2PO_4 10:90 containing 5 mM sodium 1-hexanesulfonate
Column temperature: 40
Flow rate: 0.6
Injection volume: 30
Detector: F ex 400 em 460 following post-column reaction. The column effluent mixed with the reagent pumped at 0.6 mL/min and the mixture flowed through a 10 m × 0.25 mm ID stainless steel coil at 40° to the detector. (Prepare reagent by dissolving 50 g NaOH and 800 mg potassium ferricyanide in 1 L water, store in the dark, discard after 24 h.)

CHROMATOGRAM

Retention time: 13
Internal standard: beclotiamine (3-[(4-amino-2-methyl-5-pyrimidinyl)methyl-5-(2-chloroethyl)-4-methylthiazolium chloride, Sankyo, Tokyo) (12)
Limit of detection: 2 ng/mL
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites, thiamine
Noninterfering: ethopabate

KEY WORDS

post-column reaction; chicken; plasma; pharmacokinetics

REFERENCE

Hamamoto,K.; Koike,R.; Shirakura,A.; Sasaki,N.; Machida,Y. Rapid and sensitive determination of amprolium in chicken plasma by high-performance liquid chromatography with post-column reaction, *J.Chromatogr.B*, **1997**, *693*, 489–492.

SAMPLE

Matrix: blood, tissue
Sample preparation: Slurry 6 g alumina (alumina B Akt. I, ICN Biomedicals) in MeCN:MeOH 60:40, add to a 300 × 15 column, wash with 30 mL MeCN:MeOH 60:40. Homogenize (Niti-on Bio-mixer BM-2) 5 g chopped tissue or plasma with 25 mL MeCN for 2 min, wash twice with 20 mL portions of MeCN, filter (cotton plug), wash filter with 30 mL n-hexane saturated with MeCN, add 30 g anhydrous sodium sulfate to the filtrate, let stand at room temperature for 30 min, filter (cotton plug), add 30 mL isopropanol to the filtrate. Evaporate the filtrate to dryness at 35°, reconstitute with 5 mL MeCN:MeOH 60:40, sonicate, add to the column, elute with 35 mL MeCN:MeOH 60:40. Add 10 mL

isopropanol to the eluate and evaporate it to dryness at 40°, reconstitute with 1 μg/mL chloramphenicol in mobile phase, filter (Gelman Ekikurodisk 13 CR), inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 L-column ODS (Chemicals Inspection and Testing Institute, Tokyo)
Mobile phase: MeCN:200 mM KH_2PO_4 15:85 containing 5 mM sodium 1-hexanesulfonate
Column temperature: 40
Flow rate: 0.7
Injection volume: 20
Detector: F ex 367 em 470 following post-column reaction. The column effluent mixed with the reagent pumped at 0.7 mL/min and the mixture flowed through a 10 m × 0.25 mm ID stainless steel coil at 40° to the detector. (Prepare reagent by dissolving 50 g NaOH in water, adding 800 mg potassium ferricyanide, and making up to 1 L with water.)

CHROMATOGRAM
Retention time: 8
Limit of detection: 2-4 ng/g

KEY WORDS
post-column reaction; chicken; muscle; liver; kidney; skin; plasma; SPE

REFERENCE
Takahashi,Y.; Sekiya,T.; Nishikawa,M.; Endoh,Y.S. Simultaneous high-performance liquid chromatographic determination of amprolium, ethopabate, sulfaquinoxaline, and N4-acetylsulfaquinoxaline in chicken tissues, *J.Liq.Chromatogr.*, **1994**, *17*, 4489–4512.

SAMPLE
Matrix: eggs, tissue
Sample preparation: Mix egg yolk with an equal amount of water, homogenize (Ultraturrax) for 30 s. Blend (Lameris Lab Blender 400 stomacher) 20-30 g tissue with twice the amount of water for 5 min, centrifuge at 460 g for 10 min. Dialyze (24" dialyzer with membrane Type C (Technicon, Tarrytown NY)) diluted egg yolk or tissue supernatant against water (both pumped at 0.6 mL/min), inject a 2 mL aliquot of the dialysate onto column A at 1 mL/min, wash with water at 1 mL/min for 6 min, backflush the contents of column A onto column B with mobile phase, after 2 min remove column A from the circuit, elute column B with mobile phase, monitor the effluent from column B.

HPLC VARIABLES
Column: A 50 × 4.6 37-50 μm Corasil C18; B 20 mm long LC8 (Supelco) + 150 × 4.6 5 μm Supelcosil LC8-DB
Mobile phase: MeOH:water:acetic acid:triethylamine 25:75:1:0.5 containing 5 mM heptanesulfonate
Column temperature: 40
Flow rate: 1
Injection volume: 2000
Detector: F ex 365 em 470 following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 3 m × 0.5 mm ID knitted PTFE coil to the detector. (Prepare reagent by dissolving 25 g NaOH and 160 mg potassium ferricyanide in 100 mL water.)

CHROMATOGRAM
Retention time: 10
Limit of detection: 3 ng/g

KEY WORDS
post-column reaction; column-switching; dialysis; yolk; muscle; chicken

REFERENCE

van Leeuwen,W.; Wilhelmus van Gend,H. Determination of amprolium in egg yolk and muscle tissue (chicken) by HPLC with post-column reaction and fluorometric detection, using on-line sample clean-up and pre-concentration steps, *Z.Lebensm.Unters.Forsch.*, **1988**, *186*, 500–504.

SAMPLE

Matrix: feed, premix

Sample preparation: Feed. 1 g Feed + 2 mL 5 μg/mL thiamine monophosphate + 10 mL 5% sulfosalicylic acid + 10 mL hexane, vortex for 1 min, centrifuge at 2400 g for 10 min. Remove the aqueous layer and filter it (0.45 μm, Gelman Acro LC 13). Inject a 360 μL aliquot onto a 300 × 6 glass column packed with 200-400 mesh Dowex AG 2-X8 anion exchange resin, elute with 100 mM HCl at 1.2 mL/min, after about 2 min collect a 6-10 mL fraction, neutralize with 1 M NaOH (pH 7.0 ± 0.5), inject a 500 μL aliquot of this fraction (J.Agric.Food Chem. 1980, 28, 1145). After 15-20 samples clean the sulfosalicylic acid from the column by backflushing with 700 mM NaCl containing 100 mM HCl and 2% ferric chloride at 1.2 mL/min, flush for 20-30 min after the last of the iron chelate has gone (about 3 h). Re-equilibrate for 30 min. Premix. 0.8 mg Premix + 0.1 mg pyrithiamine + 10 mL water, grind (Omni-Mixer), add 10 mL hexane, grind for 5 min, centrifuge at 2400 g at 4° for 10 min. Filter the aqueous layer (0.45 μm, Millipore), dilute 20 times, inject an aliquot.

HPLC VARIABLES

Guard column: 30 × 4.6 5 μm Rainin RP-18 guard column

Column: 30 × 3 3 μm Perkin-Elmer C18

Mobile phase: Feed. Gradient. 100 mM pH 5.5 sodium phosphate buffer for 6 min then 100 mM pH 2.6 sodium phosphate buffer for 19 min, re-equilibrate with original buffer for 15 min. Premix. Isocratic. 100 mM pH 2.6 Sodium phosphate buffer.

Flow rate: 1

Injection volume: 10

Detector: F ex 339 em 432, following post-column reaction with 0.01% potassium ferricyanide in 15% NaOH pumped at 1 mL/min. The reagent is mixed with the column effluent and passed through a 7 m × 0.4 mm i.d. PTFE tube, kept at 32°, to the detector.

CHROMATOGRAM

Retention time: 13.98 (feed), 4.03 (premix)

Internal standard: thiamine monophosphate (5.26), pyrithiamine (1.62)

OTHER SUBSTANCES

Simultaneous: thiamine diphosphate, thiamine

REFERENCE

Vanderslice,J.T.; Huang,M.-H.A. Liquid chromatographic determination of amprolium in poultry feed and premixes using postcolumn chemistry with fluorometric detection, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 920–922.

SAMPLE

Matrix: feed, premix

Sample preparation: Weigh out 4-12 g feed, add 100 mL MeOH:water 2:1 containing 5 mM sodium dioctylsulfosuccinate and 10 mM calcium chloride, shake mechanically for 1 h, centrifuge, force 8-10 mL solution through a Sep-Pak alumina A SPE cartridge, discard first 3 mL effluent, collect next 4-6 mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 mm long 5 μm Econosphere C18 or 250 × 4.6 10 μm Whatman ODS-3

Mobile phase: MeCN:water 40:60 containing 4 mM sodium dioctylsulfosuccinate, 0.3% diethylamine, and 1% acetic acid

Flow rate: 1.5

Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 12
Limit of quantitation: 2500 ng/mL

OTHER SUBSTANCES
Noninterfering: ethopabate, sulfonamides, arsanilic acid, penicillin, streptomycin, chlortetracycline

KEY WORDS
rugged; SPE

REFERENCE
Kentzer,E.J.; Cottingham,L.S.; Smallidge,R.L. Ion-pair reverse-phase liquid chromatographic determination of amprolium in complete feeds and premixes, *J.Assoc.Off.Anal.Chem.*, **1988**, *71*, 251–255.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax TP 18/10) 3 g tissue with 1 mL water and 4 mL acetone for 6 s, centrifuge at 5000 rpm for 3 min. Transfer 4 mL supernatant, add 5 mL dichloromethane, mix for 5 s, centrifuge at 3000 rpm for 3 min. Transfer the upper (water) layer to another tube and add 1 g NaCl, 3 mL MeCN, and 1 mL 300 mM NaOH. Shake vigorously for 20 s, centrifuge at 3000 rpm for 2 min, transfer the upper layer to another tube. Extract the remainder twice with 3 mL MeCN, discard the water layer. Evaporate the MeCN layers to dryness at 60° under a stream of nitrogen. Dissolve residue in 500 μL 20 mM KH_2PO_4 and filter (Spin-X) while centrifuging at 5600 g for 3 min.. Inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-ABZ
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ
Mobile phase: MeCN:buffer 10:90 (Prepare buffer by dissolving 27.2 g KH_2PO_4 and 0.94 g hexane sulfonic acid sodium salt in 750 mL water, make up to 1 L with water.)
Flow rate: 0.8
Injection volume: 20
Detector: F ex 365 em 470 following post-column reaction. The column effluent mixed in a vortex mixer with reagent pumped at 0.7 mL/min and this mixture flowed through a 10 m × 0.3 mm ID PTFE coil illuminated in a Beam Boost Photochemical Reactor to the detector. (The reagent was 1.25 M NaOH containing 25 mM potassium ferricyanide.)

CHROMATOGRAM
Retention time: 5.2
Limit of quantitation: 5 ng/g

KEY WORDS
chicken; muscle; post-column reaction; post-column photochemical derivatization

REFERENCE
Hormazabal,V.; Yndestad,M. Rapid assay for the determination of residues of amprolium and ethopabate in chicken meat by HPLC, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2517–2525.

SAMPLE
Matrix: tissue
Sample preparation: Prepare a cleanup column by plugging a 10 mm i.d. column with glass wool and adding 5 g activity I alumina, prewash with 70 mL MeCN:MeOH 90:10.

Homogenize 10 g minced chicken muscle with 50 mL MeOH at maximum speed (Ultra-Turrax T-18), filter through cotton, repeat extraction with another 50 mL MeOH. Combine filtrates, add 20 mL 1-propanol, concentrate to 3-4 mL under vacuum at 45°. Add the residue to 20 mL MeCN and 50 mL n-hexane, shake vigorously by hand for 5 s, discard n-hexane layer, add another 50 mL n-hexane, shake for 5 min on a mechanical shaker, discard n-hexane layer. Evaporate the lower phase to dryness under vacuum at 45°, dissolve the residue in 1 mL MeOH, add to cleanup column, wash with 30 mL MeCN:MeOH 90:10, elute with 30 mL MeCN:water 95:5. Evaporate the eluate to dryness under vacuum at 45°, reconstitute the residue in 1 mL water, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 7 μm LiChrosorb RP-8
Mobile phase: MeCN:200 mM KH_2PO_4 20:80 containing 5 mM sodium 1-hexanesulfonate
Column temperature: 30
Flow rate: 0.7
Injection volume: 10
Detector: F ex 367 em 470 following post-column reaction with 25 g NaOH and 0.4 g potassium ferricyanide in 500 mL water pumped at 0.7 mL/min. The column effluent and reagent were mixed and flowed through a 3 m × 0.3 mm i.d. stainless steel reaction coil to the detector.

CHROMATOGRAM

Retention time: 10
Limit of detection: 0.01 ppm

OTHER SUBSTANCES

Noninterfering: ethopabate, sulfonamides

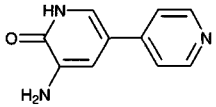
KEY WORDS

chicken; muscle; SPE

REFERENCE

Nagata,T.; Saeki,M. Liquid chromatographic determination of amprolium in chicken tissues, using post-column reaction and fluorometric detection, *J.Assoc.Off.Anal.Chem.*, **1986**, *69*, 941–943.

Amrinone



Molecular formula: $C_{10}H_9N_3O$
Molecular weight: 187.20
CAS Registry No.: 60719-84-8
Merck Index: 634
Lednicer No.: 3 147; 4 90, 115, 163

SAMPLE

Matrix: blood
Sample preparation: Mix plasma or serum (minimum 50 μL) with an equal volume MeCN, vortex for 15 s. Incubate at ambient temperature for 15 min, vortex for 15 s, centrifuge at 39 000 g for 2 min, inject a 25 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 2.1 5 μm C18 narrow-bore (Hewlett-Packard)
Mobile phase: Gradient. A was MeCN. B was 100 mm pH 6.0 phosphate buffer. A:B from 5:95 to 10:90 over 13.5 min. (Buffer was prepared by mixing 2 L 100 mm monobasic sodium phosphate with 280 mL 100 mM dibasic sodium phosphate.)
Flow rate: 0.4
Injection volume: 25
Detector: UV 320, UV 345

CHROMATOGRAM

Retention time: 3.7
Limit of detection: 100 μg/L (320 nm), 500 μg/L (345 nm)

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: acetaminophen, bumetanide, carbamazepine, cefamandole, cefonicid, cefotaxime, cefoxitin, cefprozil, ceftazidime, ceftriaxone, ceftibuten, ceftizoxime, cefuroxime, cephalexin, cephalothin, desipramine, diazepam, digoxin, dobutamine, dopamine, doxepin, epinephrine, ethosuximide, fentanyl, furosemide, gentamicin, imipramine, methotrexate, midazolam, morphine, norepinephrine, phenobarbital, phenytoin, primidone, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin (Monitoring at 345 nm may be necessary to avoid interference by cephalosporins.)

KEY WORDS

plasma; serum

REFERENCE

Pappas,J.B.; Allen,E.M.; Ross,M.; Banner,W.,Jr. HPLC micromethod for amrinone and metabolites in patients receiving concurrent cephalosporin therapy, *Clin.Chem.*, **1996**, *42*, 761–765.

SAMPLE

Matrix: blood
Sample preparation: Extract serum.

HPLC VARIABLES

Column: 150 × 3.3 7 μm Separon SGX
Mobile phase: 80 mM ammonium perchlorate in MeOH
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: k' 0.89

OTHER SUBSTANCES
Simultaneous: nicotine, strychnine, neostigmine

KEY WORDS
serum

REFERENCE
Eigendorf,H.G.; Nagel,S. Zur Analytik von Amrinone (Cordemcura). 2. Mitteillung: Hochdruckglüssig-chromatographie [The analysis of amrinone (Cordemcura). 2. High pressure liquid chromatography], *Pharmazie*, **1987**, *42*, 631–631.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL ethyl acetate, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove the organic phase and evaporate it under nitrogen at 30°, reconstitute the residue in 100 μL MeCN:5 mM pH 3.2 phosphate buffer 1:1, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: Guard-pak cyano (Waters)
Column: 100 × 8 4 μm Nova Pak cyano
Mobile phase: MeCN:5 mM pH 3.2 phosphate buffer 70:30
Flow rate: 2
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 4.18
Limit of quantitation: 250 ng/mL

KEY WORDS
plasma

REFERENCE
Bansal,R.; Louridas,A.T.; Gottesman,R.D.; Aranda,J.V. Determination of amrinone in human plasma by high-performance liquid chromatography with ultraviolet detection, *J.Liq.Chromatogr.*, **1994**, *17*, 3531–3539.

SAMPLE
Matrix: formulations
Sample preparation: Dilute a 1 mL sample to 10 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS III C18
Mobile phase: MeOH:water:0.5 M borate 40:58:2, pH 7.0
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 3

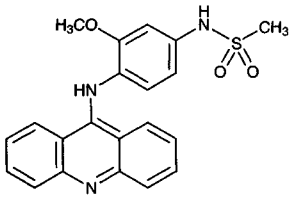
OTHER SUBSTANCES
Noninterfering: digoxin

KEY WORDS
injections; stability-indicating; 5% dextrose; 0.45% NaCl

REFERENCE

Riley,C.M.; Junkin,P. Stability of amrinone and digoxin, procainamide hydrochloride, propranolol hydrochloride, sodium bicarbonate, potassium chloride, or verapamil hydrochloride in intravenous admixtures, *Am.J.Hosp.Pharm.*, **1991**, *48*, 1245–1252.

Amsacrine



Molecular formula: $C_{21}H_{19}N_3O_3S$
Molecular weight: 393.47
CAS Registry No.: 51264-14-3
Merck Index: 635

SAMPLE

Matrix: blood
Sample preparation: Add 100 µL 20 µM IS in MeOH to a glass tube and evaporate the MeOH to dryness under a stream of nitrogen at 35°, add 500 µL plasma, adjust pH to 3.0-4.0 with 120 µL 500 mM HCl, vortex gently, add 5 mL hexane, shake for 20 min, centrifuge at 1720 g for 10 min. Remove the aqueous layer and adjust the pH to 9.0 with 500 µL saturated sodium tetraborate, add 6 mL diethyl ether, shake for 15 min, centrifuge at 1720 g for 15 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in 100 µL MeOH, inject a 20-40 µL aliquot.

HPLC VARIABLES

Column: 100 × 8 10 µm Radial-Pak C18 (Waters)
Mobile phase: MeCN:water:buffer 396:594:10 (Buffer was 13.9 mL triethylamine in 60 mL water, adjust pH to 3.0 with phosphoric acid, dilute to 100 mL with water.)
Flow rate: 7
Injection volume: 20-40
Detector: UV 254

CHROMATOGRAM

Retention time: 3.4
Internal standard: 4'-(3-methyl-9-acridinylamino)methanesulfonanilide (4.3)
Limit of detection: 50 nM

OTHER SUBSTANCES

Noninterfering: doxorubicin, chlorambucil, cytosine arabinoside, 5-fluorouracil, lomustine, melphalan, methotrexate, prednisolone, 6-thioguanine, vincristine, vinblastine, degradation products

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Jurlina,J.L.; Paxton,J.W. High-performance liquid-chromatographic method for the determination of 4'-(9-acridinylamino)methanesulfon-m-anisidide in plasma, *J.Chromatogr.*, **1983**, *276*, 367–374.

SAMPLE

Matrix: blood, cells
Sample preparation: 500 µL Plasma, whole blood, or cells + 100 µL 5 µg/mL IS in 5% dextrose, adjust pH to 3.0-4.0 with 500 mM HCl, extract with hexane. Remove the aqueous layer and adjust the pH to 9.0 with saturated sodium tetraborate, extract with anhydrous diethyl ether. Remove the organic layer and evaporate it to dryness under a stream of air at 37°, reconstitute the residue in 200 µL MeOH:148 mM phosphoric acid 50:10, centrifuge at 6000 g for 10 min, inject an aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 LiChrosorb RP-8
Column: 150 × 4.6 8 µm Cp-Spher C8 (Chrompack)

Mobile phase: MeCN:water:buffer 396:594:10 (Buffer was 13.9 mL triethylamine in 60 mL water, adjust pH to 3.0 with 85% phosphoric acid, dilute to 100 mL with water.)
Flow rate: 1.5
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 2.8
Internal standard: N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide (CI-921) (3.5)
Limit of detection: 6 ng/mL

KEY WORDS
whole blood; plasma; pharmacokinetics

REFERENCE
Brons,P.P.T.; Wessels,J.M.C.; Linssen,P.C.M.; Haanen,C.; Speth,P.A.J. Determination of amsacrine in human nucleated hematopoietic cells, *J.Chromatogr.*, **1987**, *422*, 175–185.

SAMPLE
Matrix: tissue
Sample preparation: 1 g Tissue + 1 mL saline, mince, homogenize (Polytron PT-10) at 27000 rpm for 10-15 min, adjust pH to 2.0 with 500 mM HCl, centrifuge at 12000 g for 10 min. Add the supernatant to six volumes n-hexane, mix thoroughly. Remove the aqueous phase and adjust its pH to 9.0 with saturated sodium borate, extract with six volumes of ethyl acetate. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in MeOH:water 90:10, inject an aliquot.

HPLC VARIABLES
Column: 300 × 4 μBondapak C18
Mobile phase: MeOH:water:5% pH 4.3 sodium phosphate 450:50:3
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 10-12
Limit of detection: 200 ng/g

KEY WORDS
bladder; liver; lymph node; kidney; adrenal; ovary; stomach; thyroid; heart; lung; testicle; muscle; fat; spleen; pancreas; colon; prostate; brain; oncocytoma

REFERENCE
Stewart,D.J.; Zhengang,G.; Lu,K.; Savaraj,N.; Feun,L.G.; Luna,M.; Benjamin,R.S.; Keating,M.J.; Loo,T.L. Human tissue distribution of 4'-(9-acridinylamino)-methanesulfon-m-anisidide (NSC 141549, AMSA), *Cancer Chemother.Pharmacol.*, **1984**, *12*, 116–119.

Amylase

Molecular formula: indeterminate

CAS Registry No.: 9000-92-4, 9000-85-5 (bacterial), 9000-90-2 (porcine), 9000-91-3 (sweet potato)

Merck Index: 640

SAMPLE

Matrix: blood

Sample preparation: Centrifuge at 100000 g for 15 min, inject a 200 μL aliquot. Alternatively, filter (0.22 μm cellulose nitrate), inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: 40 × 4 TSK SW

Column: 300 × 8 GlasPac TSK 3000 SW

Mobile phase: 10 mM pH 7.4 Phosphate buffer containing 135 mM NaCl

Flow rate: 0.8

Injection volume: 200 (titanium injector)

Detector: UV 280 or by enzyme activity

CHROMATOGRAM

Retention time: 12-16

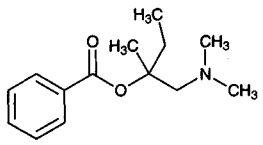
KEY WORDS

serum; GPC; SEC; human

REFERENCE

Sion,J.-P.; Laureys,M.; Gerlo,E.; Gorus,F. Detection of macroenzymes in serum by high-performance gel permeation chromatography, *J.Chromatogr.*, **1989**, *496*, 91–100.

Amylocaine



HCl

Molecular formula: $C_{14}H_{21}NO_2$
Molecular weight: 235.33
CAS Registry No.: 532-59-2, 532-59-2 (HCl)
Merck Index: 656

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben,

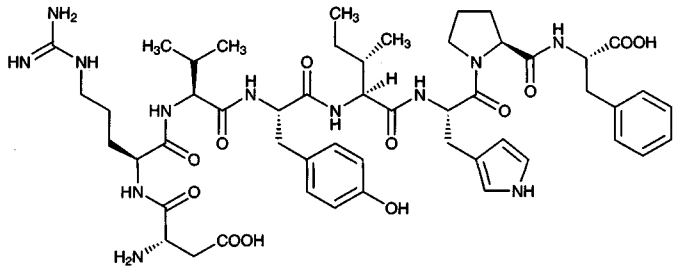
pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Angiotensin II

Molecular formula: $C_{50}H_{71}N_{13}O_{12}$
Molecular weight: 1046.19
CAS Registry No.: 4474-91-3
Merck Index: 689



SAMPLE

Matrix: blood
Sample preparation: 10 μL Serum + 240 μL 100 mM pH 7.5 potassium phosphate buffer containing 30 mM NaCl + 30 μL 1 M HCl, filter (0.2 μm), inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Spherisorb ODS-C18
Mobile phase: MeOH:0.1% trifluoroacetic acid 60:40
Column temperature: 22
Flow rate: 1.5
Injection volume: 50
Detector: UV 220

CHROMATOGRAM

Retention time: 5.6
Limit of quantitation: 0.16 nmole

OTHER SUBSTANCES

Extracted: angiotensin I

KEY WORDS

serum; rat

REFERENCE

Santos,R.A.S.; Krieger,E.M.; Greene,L.J. An improved fluorometric assay of rat serum and plasma converting enzyme, *Hypertension*, **1985**, *7*, 244–252.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL Analytichem weak cation-exchange (carboxymethylhydrogen form, CBA) SPE cartridge with 1 mL 1% trifluoroacetic acid in MeOH, 1 mL MeOH, and 2 mL water. Add 1 mL plasma to the SPE cartridge, rinse the tube with 1 mL water, add the rinse to the SPE cartridge, wash with 1 mL 1% trifluoroacetic acid in water, wash with 2 mL water, wash with 2 mL MeOH, elute with 2 mL 1% trifluoroacetic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH:buffer 50:50, inject a 5-75 μL aliquot. (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.) [Procedure was not necessarily validated for this compound.]

HPLC VARIABLES

Column: 250 × 2 5 μm Ultrasphere octyl
Mobile phase: Gradient. A was MeOH containing 10 mM sodium octanesulfonate. B was buffer containing 10 mM sodium octanesulfonate. A:B from 45:55 to 70:30 over 30 min,

maintain at 70:30 for 1 h. Alternatively isocratic at A:B 55:45 (Buffer was 5.7 g monochloroacetic acid, 2.0 g NaOH, and 0.2 g disodium EDTA in 1 L water, pH 3.2.)
Column temperature: 60
Flow rate: 0.3
Injection volume: 5-75
Detector: F ex 390 em 470 following post-column reaction. The column effluent mixed with 400 mM NaOH pumped at 0.15 mL/min and 0.05% ninhydrin pumped at 0.05 mL/min and the mixture flowed through a 12 m × 0.33 mm i.d. reaction coil at 70° to the detector.

CHROMATOGRAM
Retention time: 21 (gradient), 66 (isocratic)
Limit of detection: 100 fmole

OTHER SUBSTANCES
Simultaneous: adrenocorticotropin, angiotensin I, angiotensin III, atrial natriuretic peptide, bombesin, bradykinin, gonadorelin (LHRH), somatoliberin, vasopressin

KEY WORDS
plasma; SPE; post-column reaction

REFERENCE
Rhodes,G.R.; Boppana,V.K. High-performance liquid chromatographic analysis of arginine-containing peptides in biological fluids by means of a selective post-column reaction with fluorescence detection, *J.Chromatogr.*, **1988**, *444*, 123–131.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond Elut phenyl SPE cartridge with 1 mL MeOH and 1 mL water. 6-8 mL Blood + 500 μL inhibitor solution, cool in ice, centrifuge at 4°, add 2 mL plasma to the SPE cartridge, wash with 1 mL water, elute with 500 μL MeOH then 100 μL MeOH in to coated tubes, evaporate to dryness in a vacuum centrifuge evaporator, reconstitute with 140 μL 100 mM acetic acid, centrifuge at 4° at 3000 g for 15 min, inject a 30-100 μL aliquot of the supernatant. (Inhibitor solution was EtOH:water 2:98 containing 25 mM phenanthroline, 125 mM disodium EDTA, 2 g/L neomycin, 1 mM enalaprilat, and 10 μM pepstatin. Coat polypropylene tubes by filling with 5 g/L bovine serum albumin in 100 mM pH 7.5 Tris buffer containing 200 mg/L sodium azide overnight.)

HPLC VARIABLES
Guard column: 5 μm Lichrospher RP-18 end-capped
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:86 mM pH 3.0 triethylammonium phosphate 21:79 (After 20 injections wash column with MeCN:water 80:20 for 100 min.)
Column temperature: 45
Flow rate: 1
Injection volume: 30-100
Detector: UV 210 or immunoassay

CHROMATOGRAM
Retention time: 6.4

OTHER SUBSTANCES
Simultaneous: angiotensin III

KEY WORDS
rat; SPE; plasma

REFERENCE
Huang,H.; Baussant,T.; Reade,R.; Michel,J.B.; Corvol,P. Measurement of angiotensin II concentration in rat plasma: pathophysiological applications, *Clin.Exp.Hypertens.[A].*, **1989**, *11*, 1535–1548.

SAMPLE
Matrix: blood
Sample preparation: Add enalaprilat, 1,10-phenanthroline, and tripotassium EDTA to blood to give final concentrations of 3.6 μM, 2.5 mM, and 1.5 mg/mL respectively, separate plasma by centrifuging at 4°. 2 mL Plasma + ^{125}angiotensin II, add to a hexane-washed, conditioned 500 mg Bond Elut C8 SPE cartridge, elute with two 1 mL portions of MeCN: water 50:50 containing 0.1% trifluoroacetic acid, filter the eluate, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 Dynamax C8 (Rainin)
Mobile phase: Gradient. A was MeCN:water 20:80 containing 0.1% trifluoroacetic acid, adjusted to pH 4.0 with ammonium hydroxide. B was MeCN:water 35:65 containing 0.1% trifluoroacetic acid, adjusted to pH 4.0 with ammonium hydroxide. A:B from 100:0 to 0: 100 over 20 min.
Column temperature: 45
Flow rate: 1
Detector: RIA

CHROMATOGRAM
Retention time: 11.9
Internal standard: ^{125}angiotensin II (16)

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin III

KEY WORDS
plasma; SPE

REFERENCE
Goldberg,M.R.; Tanaka,W.; Barchowsky,A.; Bradstreet,T.E.; McCrea,J.; Lo,M.-W.; McWilliams,E.J.,Jr.; Bjornsson,T.D. Effects of losartan on blood pressure, plasma renin activity, and angiotensin II in volunteers, *Hypertension*, **1993**, *21*, 704–713.

SAMPLE
Matrix: enzyme incubations
Sample preparation: 5 μL Serum + 70 μL 200 mM pH 7.5 phosphate buffer containing 30 mM NaCl + 20 μL 800 μM angiotensin I + 10 μL water, heat at 37° for 15 min, add 100 μL 500 mM perchloric acid, centrifuge at 800 g for 5 min. Remove a 100 μL aliquot of the supernatant and cool in ice-water, add 50 μL 5 mM benzoin in 2-methoxyethanol, add 50 μL mercaptoethanol solution, add 100 μL 0.8 M KOH, heat on a boiling water bath for 1.5 min, add 100 μL 1.2 M HCl:1 M pH 8.5 Tris-HCl buffer 50:50 (?) to adjust pH to 8.5, inject a 100 μL aliquot. (Prepare mercaptoethanol solution by dissolving 780 mg β-mercaptoethanol and 2.52 g sodium sulfite in 80 mL water, make up to 100 mL with water. The procedure measures the activity of angiotensin-converting enzyme in serum. The enzyme converts angiotensin I to angiotensin II which is then determined by HPLC.)

HPLC VARIABLES
Column: 250 × 4 5 μm TSK gel ODS-120T (Toyo Soda)
Mobile phase: MeOH:48 mM pH 8.5 phosphate buffer 33:67
Flow rate: 0.8
Injection volume: 100

Detector: F ex 325 em 435

CHROMATOGRAM
Retention time: 10
Limit of detection: 80-300 fmole

OTHER SUBSTANCES
Extracted: angiotensin I

KEY WORDS
derivatization

REFERENCE
Sakamoto,Y.; Miyazaki,T.; Kai,M.; Ohkura,Y. Sensitive assay for serum angiotensin-converting enzyme and separation of angiotensin analogues by high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1986**, *380*, 313–320.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 1 mg/g solution in water.

HPLC VARIABLES
Column: 250 × 4.6 5μm Zorbax Rx C18
Mobile phase: MeCN:buffer 20:80 (Buffer was 15 mM pH 7.0 phosphoric acid containing 5 mM hexadecyltrimethylammonium bromide.)
Column temperature: 30
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin III

REFERENCE
Walker,T.A. Micellar HPLC: Investigation of the retention of positively charged peptides using cationic micellar mobile phases, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1715–1727.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: Gradient. A was 10 mM pH 4.15 ammonium acetate. B 1.5 mL/L acetic acid in MeOH. A:B from 80:20 to 25:75 over 45 min.
Flow rate: 2
Detector: UV 210

CHROMATOGRAM
Retention time: 23.0

OTHER SUBSTANCES
Simultaneous: angiotensin I, tetradecapeptide, tetrapeptide

REFERENCE

Tonnaer,J.A.D.M.; Verhoef,J.; Wiegant,V.M.; de Jong,W. Separation and quantification of angiotensins and some related peptides by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 303–309.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 1-10 μL aliquot of an aqueous solution.

HPLC VARIABLES

Guard column: 30 × 4.6 Micro-Guard ODS-10 (Bio-Rad)
Column: 150 × 4 10 μm Bio-Sil ODS-10 (Bio Rad)
Mobile phase: MeCN:50 mM NaH_2PO_4 25:75, pH 6.0
Flow rate: 1
Injection volume: 1-10
Detector: UV 210

CHROMATOGRAM

Retention time: 3.3

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin III

REFERENCE

Guy,M.N.; Roberson,G.M.; Barnes,L.D. Analysis of angiotensins I, II, III, and iodinated derivatives by high-performance liquid chromatography, *Anal.Biochem.*, **1981**, *112*, 272–277.

SAMPLE

Matrix: solutions
Sample preparation: Cool in ice while mixing 100 μL of an aqueous solution, 50 μL 5 mM benzoin in 2-methoxyethanol, 50 μL mercaptoethanol solution, and 100 μL 0.8 M KOH, heat on a boiling water bath for 1.5 min, add 100 μL 1.6 M HCl:1 M pH 8.5 Tris-HCl buffer 50:50, inject a 100 μL aliquot. (Prepare mercaptoethanol solution by dissolving 780 mg β-mercaptoethanol and 2.52 g sodium sulfite in 80 mL water, make up to 100 mL with water.)

HPLC VARIABLES

Column: 15 × 4 (sic) 5 μm LiChrosorb RP-18
Mobile phase: MeCN:50 mM pH 8.5 phosphate buffer 31:69
Flow rate: 0.8
Injection volume: 100
Detector: F ex 325 em 425

CHROMATOGRAM

Retention time: 5
Limit of detection: 27 fmole

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin III, gonadorelin, leupeptin acid, substance P, tuftsin

KEY WORDS

derivatization

REFERENCE

Kai,M.; Miyazaki,T.; Sakamoto,Y.; Ohkura,Y. Use of benzoin as pre-column fluorescence derivatization reagent for the high-performance liquid chromatography of angiotensins, *J.Chromatogr.*, **1985**, *322*, 473–477.

SAMPLE

Matrix: solutions

Sample preparation: 200 μL Solution + 100 μL chloroform + 50 μL 3 M KOH, heat at 60° for 10 min, cool in ice-water for 1 min, add 50 μL 14 M acetic acid, add 300 μL freshly prepared 265 μg/mL 1,2-diamino-4,5-dimethoxybenzene monohydrochloride in water (with cooling in ice-water), heat at 60° for 18 min, cool, inject a 100 μL aliquot. (Prepare 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as follows. Stir 483 g veratrole in 1.45 L acetic acid at 15° for 1 h, add 683 g concentrated nitric acid (d 1.05) over 1 h (maintain the temperature below 40° by cooling and regulating the rate of addition of the nitric acid). Continue stirring and add 2.127 L fuming nitric acid (d 1.50) over 1 h while maintaining the temperature below 30°, let stand for 2 h, pour into a large volume of cold water, filter, wash the solid with water until the washings are neutral, recrystallize from EtOH to give 4,5-dinitroveratrole (mp 129.5-130.5°) (J. Am. Chem. Soc. 1946, 68, 1536). Reflux 5 g 4,5-dinitroveratrole in 200 mL benzene (Caution! Benzene is a carcinogen!), add 100 g 60 mesh iron powder and 20 mL concentrated HCl in small portions over 1 h, reflux for 4 h, add 10 mL water, reflux for 2 h, cool, make alkaline with 2.5 M NaOH, extract several times with 200 mL portions of benzene. Combine the organic layers and evaporate them to dryness, add 10 mL concentrated HCl, recrystallize from EtOH to give 1,2-diamino-4,5-dimethoxybenzene monohydrochloride as very slightly pink needles (mp 240°) (Anal. Chim. Acta 1982, 134, 39).)

HPLC VARIABLES

Column: 150 × 4 5 μm LiChrosorb RP-18

Mobile phase: MeCN:buffer:50 mM sodium 1-hexanesulfonate 26:64:10 (Prepare buffer by dissolving 14.9 g KCl in 950 mL water, adjusting pH to 2.2 with concentrated HCl, and making up to 1 L with water.)

Flow rate: 0.8

Injection volume: 100

Detector: F ex 350 em 425

CHROMATOGRAM

Retention time: 14.5

Limit of detection: 11.3 pmole

OTHER SUBSTANCES

Simultaneous: angiotensin I, angiotensin III, leucine enkephalin, methionine enkephalin

KEY WORDS

derivatization; specific for tyrosine-containing peptides

REFERENCE

Ishida,J.; Kai,M.; Ohkura,Y. High-performance liquid chromatography of tyrosine-containing peptides by pre-column derivatization involving formylation followed by fluorescence reaction with 1,2-diamino-4,5-dimethoxybenzene, *J.Chromatogr.*, **1986**, *356*, 171–177.

SAMPLE

Matrix: solutions

Sample preparation: Inject a 10-50 μL aliquot.

HPLC VARIABLES

Column: 200 × 4 5 μm TSKgel ODS-120T (Toyo Soda)

Mobile phase: Gradient. A was MeCN:200 mM pH 2.3 sodium phosphate buffer 5:95. B was MeCN:200 mM pH 2.3 sodium phosphate buffer 40:60. A:B 100:0 for 5 min, to 68.6:31.4 step gradient), to 35.7:64.3 over 45 min.

Flow rate: 1

Injection volume: 10-50

Detector: F ex 325 em 435 following post-column reaction. The column effluent mixed with reagent A pumped at 1 mL/min and the mixture flowed through a 15 m × 0.3 mm ID

PTFE coil at 76°. The effluent from this coil mixed with reagent B pumped at 0.4 mL/min and this mixture passed through a 10 × 4 column packed with 40 mg glass wool. (Prepare reagent A by mixing equal volumes of 6 mM benzoin in 2-methoxyethanol, 4.8 M KOH, and 2.1 M β-mercaptoethanol. Prepare reagent B by mixing equal volumes of 1 M Tris and 4.2 M HCl.)

CHROMATOGRAM
Retention time: 20
Limit of detection: 5-15 pmole

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin III, kallidin, kyotorphin, β-melanocyte stimulating hormone, substance P
Noninterfering: estriol, estrone-3-sulfate, methionine enkephalin, phenylalanine, phenylpyruvic acid, propionic acid, sorbic acid, tryptophan, tyrosine

KEY WORDS
post-column reaction

REFERENCE
Ohno,M.; Kai,M.; Ohkura,Y. On-line post-column fluorescence derivatization of arginine-containing peptides in high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *392*, 309–316.

SAMPLE
Matrix: solutions
Sample preparation: Dry a 5 μL aliquot of a 0.1-4 mM aqueous solution, reconstitute with 100 μL pH 9.5 100 mM lithium carbonate/sodium bicarbonate buffer, add 100 μL 15 mM 9-fluorenylmethyl chloroformate in acetone, let stand at room temperature for 30 s, wash with five 500 μL portions of n-pentane. Remove a 10 μL aliquot of the aqueous phase and add it to 90 μL 10 mM NaH_2PO_4, mix, inject a 2 μL aliquot.

HPLC VARIABLES
Column: 125 × 2 5 μm LiChrospher RP-2
Mobile phase: MeCN:10 mM pH 4.9 NaH_2PO_4 54:46
Flow rate: 1
Injection volume: 2
Detector: F ex 260 em 310

CHROMATOGRAM
Retention time: 5.2
Limit of detection: 500 fmole

KEY WORDS
derivatization

REFERENCE
Vogt,W.; Egeler,E.; Sommer,W.; Eisenbeiss,F.; Meyer,H.D. High-performance liquid chromatographic determination of hormonal peptides and their fluorenylmethoxycarbonyl derivatives, *J.Chromatogr.*, **1987**, *400*, 83–89.

SAMPLE
Matrix: solutions
Sample preparation: Mix a 20 μL aliquot with 20 μL 500 mM pH 9.0 potassium phosphate buffer and 30 μL 2 mg/mL fluorescamine, mix, let stand at room temperature for 5 min, inject a 10-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 2 5 μm Ultrasphere octylsilica

Mobile phase: Gradient. MeOH:buffer from 40:60 to 65:35 over 10 min, maintain at 65:35 for 10 min. (Prepare buffer by dissolving 1.21 g Tris and 2.8 mL triethylamine in 1 L water, adjust pH to 7.0 with phosphoric acid.)
Column temperature: 50
Flow rate: 0.3
Injection volume: 10-50
Detector: F ex 390 em 470 (cutoff filter)

CHROMATOGRAM
Retention time: 10

OTHER SUBSTANCES
Simultaneous: angiotensin I, angiotensin III

KEY WORDS
derivatization

REFERENCE
Boppana,V.K.; Miller-Stein,C.; Politowski,J.F.; Rhodes,G.R. High-performance liquid chromatographic determination of peptides in biological fluids by automated pre-column fluorescence derivatization with fluorescamine, *J.Chromatogr.*, **1991**, *548*, 319–327.

SAMPLE
Matrix: solutions
Sample preparation: Mix 20 μL of a 25 μM peptide solution in water with 50 μL 200 mM ascorbic acid in water, 100 μL 10 mM NaCN in water, 200 μL 5 mM naphthalene-2,3-dicarboxaldehyde in MeCN, and 540 μL 100 mM pH 7.0 phosphate buffer, mix, let stand at 0-4° for 20 min, add 50 μL 200 mM taurine in water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS Hypersil
Mobile phase: THF:100 mM pH 6.0 phosphate buffer 19:81 containing 30 mM sodium 1-octanesulfonate
Column temperature: 40 ± 0.1
Flow rate: 1-2
Detector: F ex 420 em 490

CHROMATOGRAM
Retention time: k' 11
Limit of detection: 50-100 fmole

OTHER SUBSTANCES
Simultaneous: similar peptides

KEY WORDS
derivatization

REFERENCE
Patel,H.B.; Stobaugh,J.F.; Riley,C.M. Reversed-phase ion-pair liquid chromatography of the angiotensins, *J.Chromatogr.*, **1991**, *536*, 357–370.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.5 5 μm Kromasil C8 (Eka-Nobel)

Mobile phase: Gradient. A was MeCN:water 10:90 containing 0.1% trifluoroacetic acid. B was MeCN:water 90:10 containing 0.1% trifluoroacetic acid. A:B from 0:100 to 75:25 over 8 min, to 25:75 over 12 min.
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 8.7

OTHER SUBSTANCES
Simultaneous: angiotensin I, bradykinin, insulin, leucin enkephalin, lysozyme, melittin, methionine enkephalin, oxytocin

REFERENCE
Supelco Catalog, **1992,** p. 104.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 75 × 4.6 5 μm Hypersil WP 300-octyl
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in n-propanol. A:B 100:0 for 2.5 min, to 90:10 over 2.5 min, to 10:90 over 112 min.
Flow rate: 1
Detector: UV 225

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: bombesin, bovine growth hormone, bovine serum albumin, catalase, lysozyme, L-tryptophan

REFERENCE
Supelco Catalog, **1993,** p. 602.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 TSKgel ODS-120T
Mobile phase: Gradient. A was MeOH:water 20:80 containing 0.05% trifluoroacetic acid. B was MeOH:water 50:50 containing 0.05% trifluoroacetic acid. A:B from 100:0 to 0:100 over 1 h.
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: 12

OTHER SUBSTANCES
Simultaneous: angiotensin I, calcitonin (human), α-endorphin, β-endorphin, gonadorelin (LH-RH), protirelin (TRH), somatostatin

REFERENCE
Varian Catalog, **1993,** p. 182.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Vydac 228TP
Mobile phase: Gradient. A was 0.25% trifluoroacetic acid in water. B was 0.25% trifluoroacetic acid in MeCN:water 70:30. A:B from 95:5 to 0:100 over 30 min.
Flow rate: 1.5
Detector: UV 220

CHROMATOGRAM
Retention time: 11

OTHER SUBSTANCES
Simultaneous: angiotensin I, bradykinin, eledosin, insulin, lysozyme, myoglobin, neurotensin, ovalbumin, oxytocin

REFERENCE
Supelco Catalog, **1993,** p. 581.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mM solution in 100 mM pH 6 morpholinoethanesulfonic acid/NaOH buffer. Remove a 20 μL aliquot and add it to 5 μL 25 mM 5-carboxytetramethylrhodamine succinimidyl ester (Molecular Probes, Eugene OR) in DMF, let stand at room temperature overnight, add 50 μL 1 M pH 8 Tris-HCl buffer, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: reversed-phase
Column: reversed-phase
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 55:45 "applied just after application of a sample".
Flow rate: 1
Injection volume: 5
Detector: UV 280

CHROMATOGRAM
Retention time: 38

OTHER SUBSTANCES
Simultaneous: angiotensin I

KEY WORDS
derivatization

REFERENCE
Shimura,K.; Kasai,K.-I. Fluorescence-labeled peptides as isoelectric point (p*I*) markers in capillary isoelectric focusing with fluorescence detection, *Electrophoresis*, **1995**, *16*, 1479–1484.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax 300 Å SB-C3

Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 5:95:0.1. B was MeCN:water:trifluoroacetic acid 5:95:0.085. A:B from 85:15 to 47:53 over 20 min.
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 215

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: carbonic anhydrase, cytochrome C, insulin, leucine enkephalin, lysozyme, myoglobin, RNAase

REFERENCE
Ricker,R.D.; Sandoval,L.A.; Permar,B.J.; Boyes,B.E. Improved reversed-phase high performance liquid chromatography columns for biopharmaceutical analysis, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 93–105.

SAMPLE
Matrix: tissue
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 10 mL MeOH, 10 mL THF, 10 mL hexane, 10 mL MeOH, and 10 mL water. Homogenize (Polytron) tissue with 20 volumes of ice-cold EtOH:180 mM HCl 75:25, centrifuge at 4° at 32570 g for 20 min, store the supernatant at -20° for 18-20 h, centrifuge at 4° at 2200 g for 20 min. Adjust the pH of the supernatant to 6.5 with 1 M NaOH, store at 4° for 1 h, centrifuge at 4° at 2200 g for 20 min, evaporate to dryness under reduced pressure, reconstitute with 10 mL pH 3 0.1% trifluoroacetic acid, add to the SPE cartridge, wash with water, wash with 10 mL MeCN:0.1% trifluoroacetic acid 10:90, elute with 10 mL MeCN:0.1% trifluoroacetic acid 30:70, inject a 50-200 μL aliquot of the eluate.

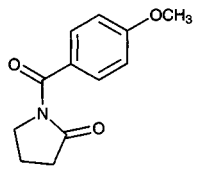
HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova Pak C18
Mobile phase: Gradient. A was MeCN:25 mM pH 7.6 phosphate buffer 5:95. B was MeCN:25 mM pH 7.6 phosphate buffer 95:5. A:B from 89:11 to 68:32 over 12 min (Waters curve 7).
Flow rate: 1.5
Injection volume: 50-200
Detector: UV 214 or radioimmunoassay

CHROMATOGRAM
Retention time: 8

KEY WORDS
rat; adrenal; brain; heart; kidney; liver; lung; ovary; plasma; testes; uterus; SPE

REFERENCE
De Silva,P.E.; Husain,A.; Smeby,R.R.; Khairallah,P.A. Measurement of immunoreactive angiotensin peptides in rat tissues: some pitfalls in angiotensin II analysis, *Anal.Biochem.*, **1988**, *174*, 80–87.

Aniracetam



Molecular formula: $C_{12}H_{13}NO_3$
Molecular weight: 219.24
CAS Registry No.: 72432-10-1
Merck Index: 700

SAMPLE
Matrix: blood
Sample preparation: 50 μL Plasma, 100 μL 50 μ g/mL IS in MeCN, mix, centrifuge at 12000 rpm for 10 min. Filter (0.45 μ m) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES
Column: 150 × 4.6 Inertsil ODS
Mobile phase: MeCN:water:acetic acid 25:75:0.5
Flow rate: 1.0
Detector: UV 254

CHROMATOGRAM
Internal standard: aspirin
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Ogiso,T.; Iwaki,M.; Tanino,T.; Ikeda,K.; Paku,T.; Horibe,Y.; Suzuki,H. Pharmacokinetics of aniracetam and its metabolites in rats, *J.Pharm.Sci.*, **1998**, *87*, 594–598.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 25 μL 5 μg/mL IS in water, mix thoroughly. Maintain sample at 4° until injection, inject 200 μL plasma onto column A with mobile phase A, after 4 min backflush the contents of column A onto column B with mobile phase B, after 1 min remove column A from the circuit, monitor the effluent from column B. Wash column A with MeOH for 10.8 min, re-equilibrate with water for 10 min. At the end of the separation backflush column A with mobile phase B for 12 min.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS; B 30 × 4.6 10 μm Brownlee RP-2 + 60 × 4 3 μm Hypersil ODS
Mobile phase: A water; B MeOH:MeCN:water 30:10:70
Flow rate: A 2; B 1
Injection volume: 200
Detector: UV 282

CHROMATOGRAM
Retention time: 8.3
Internal standard: 1-(p-ethoxybenzoyl)-2-pyrrolidinone (Ro 13-8606) (11.8)
Limit of quantitation: 5 ng/mL

KEY WORDS
plasma; column switching

REFERENCE

Guenzi,A.; Zanetti,M. Determination of aniracetam and its main metabolite, N-anisoyl-GABA, in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *530*, 397–406.

Anistreplase

Molecular formula: indeterminate
CAS Registry No.: 81669-57-0
Merck Index: 712

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Bakerbond C4
Mobile phase: Gradient. MeCN:0.1% trifluoroacetic acid from 0:100 to 60:40 over 90 min
Flow rate: 1
Injection volume: 200
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: alteplase, streptokinase, urokinase

REFERENCE
Werner,R.G.; Bassarab,S.; Hoffmann,H.; Schlüter,M. Quality aspects of fibrinolytic agents based on biochemical characterization, *Arzneimittelforschung*, **1991**, *41*, 1196–1200.